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ORIGINAL ARTICLE

A phase I, pharmacokinetic and pharmacodynamic study of nimotuzumab in Japanese patients with advanced solid tumors

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Abstract
Purpose Nimotuzumab is a humanized IgG_1 monoclonal antibody to the epidermal growth factor receptor (EGFR) and has demonstrated the absence of severe dermatological toxicity commonly caused by other EGFR-targeting antibodies. We conducted a phase I study to assess toxicities, pharmacokinetics, pharmacodynamics, and predictive biomarkers of nimotuzumab administered in Japanese patients with advanced solid tumors.
Methods Three dose levels, 100, 200, and 400 mg, of weekly i.v. nimotuzumab were given until disease progression or drug intolerability. Four patients with solid tumors were enrolled in each dose level. The expression and gene copy number of *EGFR* or its downstream transducers were investigated using skin biopsy samples and tumor specimens.
Results Planned dose escalation was completed without dose-limiting toxicity, and maximum tolerated dose was not reached. No allergic reaction and hypomagnesaemia were observed, and grade 3 or 4 toxicity did not occur. The common toxicity was skin rash (58 %); however, all of them were grade 1 or 2. In skin biopsies, no correlation was shown between doses and the phosphorylation of EGFR or its downstream signal transducers. Of 11 evaluable patients, no objective response was obtained, while 8 patients had stable disease (73 %). Patients with a higher-*EGFR* gene copy number level measured by FISH showed a longer time to progression.
Conclusions Nimotuzumab administered weekly was feasible and well tolerated up to 400 mg in Japanese patients. A low dermatological toxicity could be a notable advantage as anti-EGFR mAb, and further evaluation is warranted.

Keywords Nimotuzumab · EGFR · Phase 1 · Pharmacokinetics · Solid tumor

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Introduction

Epidermal growth factor receptor (EGFR) has become one of the most widely explored targets for anticancer therapy, because EGFR overexpression is implicated in tumor cell proliferation, invasion, angiogenesis, and metastasis [1, 2].

Several EGFR antagonists including monoclonal antibodies and small tyrosine kinase inhibitors have been investigated in colorectal cancer, head and neck cancer, and non-small cell lung cancer. However, these agents also

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act on normal human epithelial cells with a comparatively higher appearance of EGFR. As a result, dermatological toxicity including acneiform skin rash could compromise patient's quality of life (QOL) and worsen the compliance of treatment [3–5].

Nimotuzumab is a recombinant humanized monoclonal antibody against human EGFR, which is a human IgG_1. It has demonstrated blocking ability against the binding of EGF and TGF-alpha to EGFR, and also cytotoxic activity through antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC). In non-clinical studies, inhibition activity on tumor cell growth, angiogenesis, and apoptosis has been observed [6–8]. Nimotuzumab has shown clinical efficacy in head and neck cancer or glioma as combination therapy with radiotherapy [9–13]. In these clinical studies, nimotuzumab has demonstrated low frequency or absence of severe dermatological toxicity. This unique safety profile could be expected to contribute to the better QOL for patients.

The reason for low frequency of dermatological toxicity has been investigated in several recent researches [14, 15]. According to the findings, the possible explanation of low frequency of dermatological toxicity is regarded as (1) its intermediate affinity ($Kd = 10^{-8}$ M), which is at least one order lower magnitude than cetuximab or panitumumab, and (2) the difference in binding profile, where nimotuzumab requires bivalent binding for stable attachment to the cellular surface in contrast to cetuximab requiring only monovalent binding. Due to these properties, nimotuzumab could attach to EGFR only when the EGFR surface density is high enough to allow bivalent binding. EGFR is commonly overexpressed on tumor cells compared with the expression levels on normal cells; consequently, nimotuzumab could selectively attach to tumor cells without binding to normal cells.

In a previous phase I study in Canada, the tolerability of nimotuzumab was investigated up to 800 mg dose per week and did not reach MTD though one DLT was observed in the 100 mg group [16]. Subsequently, several trials demonstrated the efficacy of nimotuzumab administered 200 mg weekly to the patients with head and neck squamous cell cancer, glioma, and non-small cell lung cancer. The dose in clinical use has been set as 200 mg per week in combination with radiotherapy in several countries. According to the clinical dose in other countries, we evaluated the safety and pharmacokinetics profile of nimotuzumab at the dose levels of 100, 200, and 400 mg weekly in Japanese patients with advanced solid tumors. The secondary objectives were to assess tumor response, pharmacodynamic (PD) effects using skin tissue, and biomarkers to predict the clinical efficacy of nimotuzumab.

Methods

Patient eligibility

Patients with histologically or cytologically confirmed advanced solid tumors either refractory to standard therapy or for which no effective standard treatment existed were eligible if they fulfilled all of the following criteria: age between 20 and 75 years; ECOG performance status (PS) of 0–1; life expectancy of $\geq$3 months; adequate bone marrow, liver, and renal functions; and arterial oxygen pressure (PaO_2) $\geq$70 mmHg. Exclusion criteria included previous exposure to EGFR-targeted antibodies; brain metastases that required systemic medication; obvious pneumonitis or pulmonary fibrosis confirmed by chest CT (computed tomography).

The protocol was approved by the independent ethical committee at each center and carried out according to the principles of the Declaration of Helsinki and Good Clinical Practice guidelines. All patients gave written informed consent before study entry.

Written informed consent for the pharmacodynamics and biomarker analysis was additionally obtained from those who submitted their tumor and skin biopsy samples.

Screening and study assessments

Physical examination, radiographic tumor assessment, ECG, and chest CT investigation were performed within 28 days prior to the first dose of nimotuzumab. A pregnancy test was checked from women with childbearing potential.

Nimotuzumab was administered intravenously every week over 30 min without pre-medication for preventing allergic reaction. Each cycle was defined as 4 weeks, and treatment was continued until disease progression, occurrence of intolerable toxicity, or patient's withdrawal of consent.

Before each cycle, physical examination including vital signs, ECOG PS, blood tests including complete blood cell count and biochemistry including KL-6 were conducted. Toxicities were assessed according to the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) version 3.0. Tumor response was assessed after 4 weeks, thereafter at least every 8 weeks using Response Evaluation Criteria in Solid Tumors (RECIST) criteria version 1.0.

Treatment administration and dose escalation procedure

The initial dose of nimotuzumab was 100 mg, and the dose was escalated to 200 and 400 mg.

DLTs were initially evaluated in at least 3 patients for each dose level, and the escalation to the next dose level was implemented if less than 1/3 of the patients experienced

DLT. When one patient experienced a DLT, additional three patients were enrolled and the incidence of DLTs was evaluated in a total of six evaluable patients at each level. DLTs were defined as the following toxicities related to nimotuzumab: (1) CTCAE grade 4 fatigue, hematotoxicity, nausea, vomiting, diarrhea, or electrolyte abnormality; (2) grade 3 nausea, vomiting, or diarrhea persisting despite administration of maximum supportive care; (3) grade 3 or higher fatigue persisting for 7 days or longer; (4) any toxicity causing postponement of treatment continuously twice. DLTs were assessed during the first cycle at each dose level. The MTD was defined as the lowest dose at which 33 % or more of patients experienced DLT in the first cycle.

Pharmacokinetic analysis

Blood samples (3 mL) were collected prior to the first dosing and at 5 min, 1, 3, 8, 24, 48, and 72 h after dosing on day 1, and prior to and 5 min after dosing on days 8, 15, 22, 29, 36, and 50. Serum concentration of nimotuzumab was analyzed using the ELISA method as shown in the Online Resource 1. The lower detection limit for the serum concentration of nimotuzumab was 2 μg/mL. The pharmacokinetic (PK) parameters in the first cycle were calculated by a non-compartment analysis using the computer software SAS version 8.2 (SAS Institute, Japan) with the same analytical approach as the bolus model generated by WinNonlin Professional (5.2.1, Pharsight, Mountain View, California). The maximum concentration (C_{max}) and time to C_{max} (t_{max}) were obtained by measured values. The apparent elimination half-life ($t_{1/2}$) was obtained by linear regression of 3 or more log-transformed data points in the terminal phase. The area under the concentration versus the time curve up to the time of the last measurable concentration data ($AUC_{0–168h}$) was obtained by the trapezoidal method (linear up/log down). The AUC values were extrapolated to infinity ($AUC_{0–inf}$) using the equation $AUC_{0–168h} + C_{168h}/\lambda$, where C_{168h} is the last measurable concentration, and λ is the terminal elimination rate. The total body clearances (CL_t) were calculated by the equation $Dose/AUC_{0–inf}$.

Immunogenicity (human anti-humanized antibody)

Serum samples for the analysis on human anti-humanized antibody (HAHA) were collected prior to the first administration and at the end of the first cycle and repeated at least once every 2 cycles. HAHA was measured by the ELISA method as shown in the Online Resource 2.

Pharmacodynamic analysis using skin tissue samples

Skin biopsy was performed prior to the first administration and after fifth administration on patients who provided consent for participating in the PD analysis. Expression status of EGFR, phosphorylated EGFR (p-EGFR), AKT, phosphorylated AKT (p-AKT), MAPK, phosphorylated MAPK (p-MAPK), and Ki-67 in the skin samples were analyzed by the immunohistochemical staining. EGFR PharmDx kit (Dako, Glostrup, Denmark) was used for detection of EGFR. p-EGFR, AKT, p-AKT, MAPK, p-MAPK, and Ki-67 were detected by the primary antibodies of phospho-EGFR (Y1068) Rabbit mAb (EP774Y) (abcam), AKT1 rabbit mAb #4685 (Cell signaling, Beverly, MA, USA), phospho-AKT (Ser 473) rabbit mAb #3787 (Cell signaling), p44/42 MAP Kinase Ab #9102, phospho-p44/42 MAP Kinase (Thr202/Tyr204) Rabbit mAb #4376 (Cell signaling), and Ki-67 Ab #M7240 (Dako, Glostrup, Denmark), respectively. Protein expression levels were evaluated as positive cell rates (%) calculated by the number of positive cells per 100 cells under microscope.

Biomarker research using tumor tissue samples

Immunohistochemical staining for EGFR and its downstream signal transducers was performed with paraffin-embedded sections of tumor samples. The antibodies used for tumor tissue samples were the same as those in the PD analysis using skin biopsies. The positive cell rate (%) was calculated based on the number of positive cells per 100 tumor cells under microscope.

EGFR gene status was investigated by FISH, Paraffin pretreatment kit, LSI EGFR SpectrumOrange/CEP7 SpectrumGreen probe, DAPI I (Vysis, USA), and IGEPAL CA-630 (Sigma, USA). The numbers of *EGFR* gene and chromosome 7 centromeres were counted in 20 tumor cells and assessed for amplification or aneuploidy. Patients were classified into six FISH strata: (1) disomy (≤2 copies in >90 % of cells); (2) low trisomy (3 copies in 10–39 % of the cells); (3) high trisomy (3 copies in ≥40 % of cells); (4) low polysomy (≥4 copies in 10–39 % of cells); (5) high polysomy (≥4 copies in ≥40 % of cells); and (6) gene amplification (defined by presence of tight *EGFR* gene clusters and a ratio of *EGFR* gene to chromosome 7 centromere of ≥2 or ≥15 copies of *EGFR* per cell in ≥10 % of analyzed cells) [18, 19].

Results

Patient characteristics

From May 22, 2007, to August 1, 2008, 13 patients were enrolled in this study; however, one patient with colorectal cancer registered to the 100 mg dose level withdrew from the study before administration of nimotuzumab in order to receive radiation therapy for bone metastasis. Four patients were administered nimotuzumab at each level and included

in the safety analysis population and the MTD analysis population. Their characteristics are shown in Table 1. Of these 12 patients, the tumor types were 5 colorectal cancer, 3 non-small cell lung cancer, 2 gastric cancer, 1 renal cancer, and 1 leiomyosarcoma. All patients had received previous chemotherapy regimens, and 8 of 12 patients had received more than 4 prior regimens. Of these 12 patients, 1 patient at the 100 mg level was judged as ineligible since there had been pleural effusion requiring drainage before enrollment. Therefore, that patient was excluded from the PK analysis population and efficacy analysis population.

Table 1 Patient characteristics in safety analysis population

Dose	100 mg	200 mg	400 mg	All dose (%)
No. of treated patients	4	4	4	12
Age (years)				
Mean ± SD	53.0 ± 3.4	61.8 ± 9.4	56.8 ± 5.4	57.2 ± 7.0
Sex				
Female	1	2	4	7 (58.3 %)
Male	3	2	0	5 (41.7 %)
PS (ECOG)				
0	2	2	2	6 (50.0 %)
1	2	2	2	6 (50.0 %)
No. of prior regimen				
1	1	0	1	2 (16.7 %)
2	0	0	1	1 (8.3 %)
3	1	0	0	1 (8.3 %)
≥4	2	4	2	8 (66.7 %)
Primary tumor				
Colorectal cancer	2	2	1	5 (41.7 %)
NSCLC	1	1	1	3 (25.0 %)
Gastric cancer	0	1	1	2 (16.7 %)
Renal cancer	0	0	1	1 (8.3 %)
Leiomyosarcoma	1	0	0	1 (8.3 %)

Safety

Twelve patients had received a total of 52 cycles. The mean number of cycles was 4.3 (5.3, 2.8, and 5.0 at the dose levels of 100, 200, and 400 mg, respectively). The mean treatment period (± standard deviation) was 121.9 (± 77.5) days {147.5 (± 98.7), 79.0 (± 16.7), and 139.3 (± 90.8) days at the dose levels of 100, 200, and 400 mg, respectively}. All 12 patients discontinued nimotuzumab due to disease progression.

Table 2 presents the nimotuzumab-related adverse event during all cycles. No DLT was observed at any dose levels; therefore, the MTD was not reached up to 400 mg dose level. The most common adverse drug reaction observed in ≥15 % of patients were rash (50.0 %), anorexia (16.7 %), nausea (16.7 %), fatigue (16.7 %), aspartate aminotransferase increased (16.7 %), and blood alkaline phosphatase increased (16.7 %). As to dermatological toxicity, known as a class effect of anti-EGFR mAbs, one patient developed a grade 2 rash, whereas the other five patients of rash and one patient of dermatitis acneiform were of grade 1. No grade 3 or higher adverse drug reaction and no infusion reaction were observed at any dose levels, and there was no adverse drug reaction that led to a delay in treatment or dose reduction.

Efficacy

Eleven patients were evaluable for anti-tumor response. While complete response and partial response were not achieved in any dose levels, stable disease was observed in 8 patients (72.7 %). Median time to progression (TTP) was 97 days in eleven patients.

Pharmacokinetic analysis

Serum samples were evaluated for 11 patients. The mean serum concentration–time profile of nimotuzumab after the first administration in the first cycle is described in Fig. 1, and the PK profile of nimotuzumab is summarized in Table 3 and Fig. 2.

The serum nimotuzumab concentration after the first treatment on the first cycle reached maximum immediately after the infusion at all doses and decreased subsequently. The mean $t_{1/2}$ was 34, 47, and 75 h at a dose of 100, 200, and 400 mg, respectively.

C_{max} and AUC_{0-inf} of nimotuzumab increased in an exponential manner in the range of 100–400 mg. The mean total body clearances (CL_t) were 78.753, 47.358, and 28.960 mL/h at a dose of 100, 200 and 400 mg, respectively. The CL_t showed exponential decrease from 100 to 400 mg, and the slope of CL_t was gradual especially between 200 and 400 mg, similar to the previous PK study [17].

The serum concentration–time profile of nimotuzumab following the first administration in cycle 1 up to the fourth administration in cycle 2 is shown in Fig. 3.

Serum concentrations of nimotuzumab at each dose level increased along with the first and fourth administration and then reached plateau concentration by the fourth administration in cycle 2.

HAHA response

The serum samples were collected from 12 patients at pre-treatment, cycle 1, 3, 5, 7, and 9 as possible. Totally 43 serum samples including 12 pre-treatment and 31 on-treatment samples were measured for HAHA. However, no HAHA was detected during the treatment cycles of all patients.

Table 2 Adverse drug reaction during all cycles

Event	100 mg ($n = 4$)			200 mg ($n = 4$)			400 mg ($n = 4$)			All doses ($n = 12$)	
	Grade			Grade			Grade				
	1	2	3	1	2	3	1	2	3	*n*	(%)
Non-hematologic toxicity											
Rash	1	1		2			2			6	50.0
Nausea				1			1			2	16.7
Fatigue	1						1			2	16.7
Anorexia					1		1			2	16.7
Bradycardia							1			1	8.3
Constipation							1			1	8.3
Diarrhea	1									1	8.3
Dyspepsia							1			1	8.3
Dermatitis acneiform							1			1	8.3
Hypothermia		1								1	8.3
Hematologic toxicity											
AST increased				1			1			2	16.7
ALP increased				1				1		2	16.7
ALT increased				1						1	8.3
γ-GTP increased					1					1	8.3
Albumin increased							1			1	8.3
Creatinine increased				1						1	8.3
Potassium increased				1						1	8.3
WBC count decreased								1		1	8.3
Neutrophil count decreased								1		1	8.3

Adverse drug reaction was defined as those toxicities with 'definite', 'probable', 'possible' relativity to nimotuzumab, or 'unknown'

ALT: L-alanine aminotransferase, AST: L-aspartate aminotransferase, ALP: alkaline phosphatase, γ-GTP: γ-glutamyl transpeptidase, WBC: White blood cell

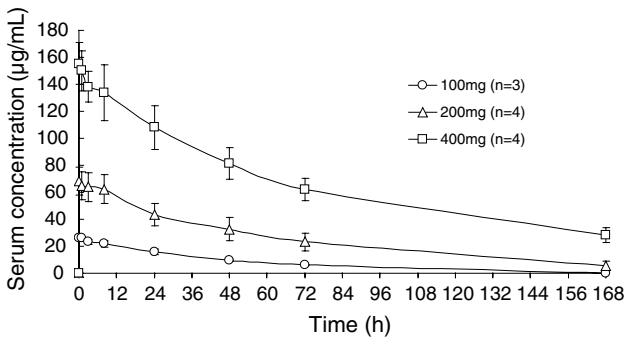

Fig. 1 Serum concentration–time profile of nimotuzumab after the first administration in cycle 1. The arithmetic mean ± arithmetic SD of each time point for each group is shown

Pharmacodynamic analysis using skin tissue

Skin samples at pre- and on-treatment were evaluated for 11 patients. The expression level of EGFR, p-EGFR, AKT, p-AKT, MAPK, p-MAPK, and Ki-67 was analyzed by immunohistochemical staining, and its positive cell rate was counted. However, these expression levels showed no certain tendency through pre- and on-treatment as shown in the table (Online Resource 3). Furthermore, no correlation was shown between the nimotuzumab doses and phosphorylation of EGFR, AKT, and MAPK.

Correlation between the markers in tumor tissue and efficacy

In order to investigate the correlation between the biomarkers and nimotuzumab efficacy, tumor tissue was obtained from 8 patients, 2, 2, and 4 patients in 100, 200, and 400 mg dose level, respectively.

The gene copy number was determined by FISH and classified into 3 categories (−; disomy, low trisomy, and high trisomy, +; low polysomy, ++; high polysomy and gene amplification). The time to progression by *EGFR* gene copy number level was shown in Fig. 4. A tendency of longer TTP was observed in patients with higher-*EGFR* gene copy number level, though the sample size was limited and the tumor type was disparate in this study.

The levels of EGFR, AKT, p-AKT, MAPK, and p-MAPK detected by immunohistochemistry (IHC) showed no relationship with clinical efficacy as shown in the table (Online Resource 4).

Table 3 Pharmacokinetic parameters after the first administration in cycle 1

Pharmacokinetic parameter	Dose		
	100 mg	200 mg	400 mg
	$n = 3$	$n = 4$	$n = 4$
C_{max} (μg/mL)	26.767 ± 1.026	68.550 ± 10.345	157.250 ± 15.064
t_{max} (h)	0.687 ± 0.535	0.050 ± 0.029	0.540 ± 0.526
$AUC_{0\text{-}inf}$ (μg h/mL)	1,271.923 ± 63.245	4,521.940 ± 1,181.627	14,107.898 ± 2,342.115
CL_t (mL/h)	78.753 ± 3.993	47.358 ± 16.074	28.960 ± 4.924
$t_{1/2}$ (h)	34.347 ± 3.332	47.498 ± 9.376	75.805 ± 6.986

Arithmetic mean ± arithmetic SD

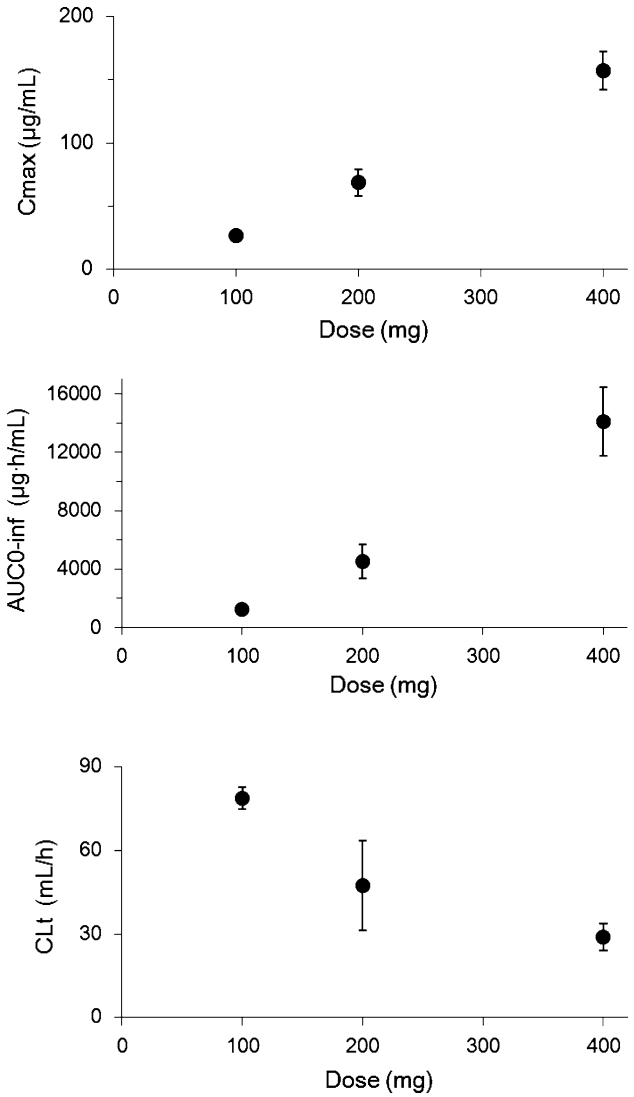

Fig. 2 Dose relationship of C_{max}, $AUC_{0\text{–}inf}$, and CL_t of nimotuzumab during the first administration in cycle 1. The arithmetic mean ± arithmetic SD 100 mg, $n = 3$; 200 mg, $n = 4$; 400 mg, $n = 4$

Discussion

This is the first study on Japanese patients with solid tumors to investigate the safety and pharmacokinetics of 100, 200, and 400 mg nimotuzumab administered weekly. All 12 patients tolerated well the treatment of nimotuzumab at a dose of 100, 200, and 400 mg, without grade 3 or higher adverse events that led to treatment delay or dose reduction. No DLT was observed in the planned dose range of 100–400 mg nimotuzumab, and MTD was not reached. Furthermore, frequencies of adverse events did not increase with higher doses of nimotuzumab as the previous phase I study conducted in Canada [16]. These results suggest the absence of a dose-dependent toxicity relationship for nimotuzumab.

In this study, there is no grade 3 or 4 dermatological toxicity, and most of the dermatological toxicity was grade 1. The incidence of rash of all grades was 50 % in each dose level, however, localized to the limited body surface, and all of them were resolved during study treatment within approximately 1 week. Consistent with our study, nimotuzumab was previously reported to cause rarely severe skin toxicity [17, 26]; however, such toxicity is commonly observed in the clinical use of other anti-EGFR mAbs, leading to increase in patient risk for infections and early termination of the treatment [4, 5]. Furthermore, we demonstrated that no hypomagnesaemia was observed, similar to other nimotuzumab phase I studies [16]. Hypomagnesaemia is known as a common adverse event with other anti-EGFR mAbs as well as platinum-based chemotherapy and considered to be related to nephrotoxicity or cardiovascular toxicity [21–23]. Taken together, the safety profile of nimotuzumab could be expected to maintain good QOL as well as compliance and shows a potential to combine nimotuzumab with other drugs which cause dermatological toxicity or hypomagnesaemia.

In PK analysis, the trough concentration of nimotuzumab reached a steady state after the fourth cycle. The trough level was approximately 15 and 55 μg/mL in 200 and 400 mg, respectively, which reached the concentration to show the cell-growth inhibitory activity in the previous in vitro study [24]. The mean $t_{1/2}$ was approximately 34, 47, and 75 h at a dose of 100, 200, and 400 mg, respectively. Nimotuzumab is a humanized mAb; however, the $t_{1/2}$ was similar to cetuximab which is a chimeric antibody to EGFR, rather than the other humanized antibody such as

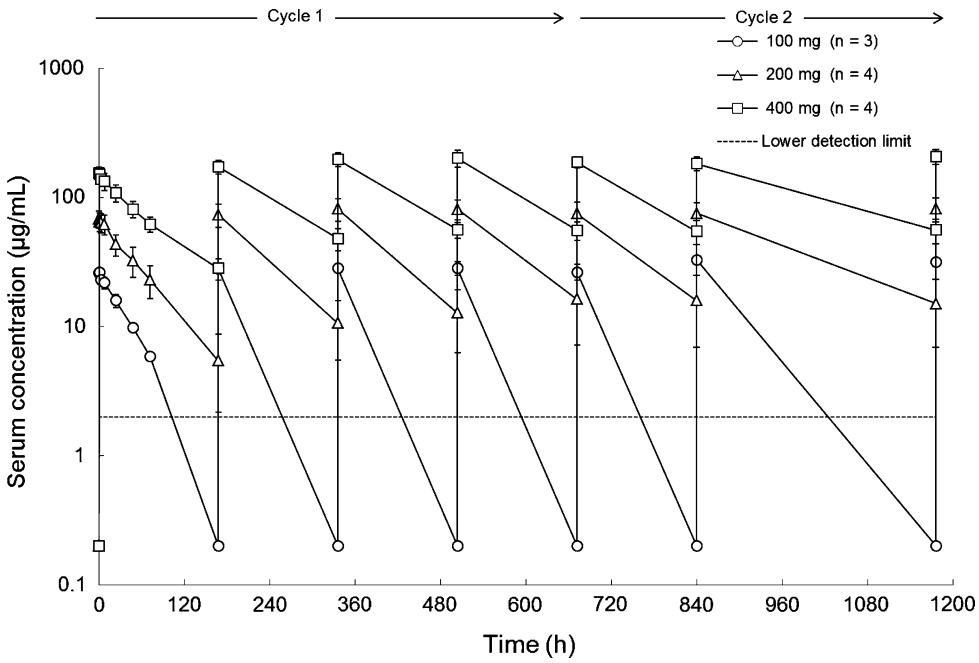

Fig. 3 Serum concentration–time profile of nimotuzumab following the first administration in cycle 1 up to the fourth administration in cycle 2. The lower detection limit for the serum concentration was 2.00 (μg/mL). If the concentration was lower than 2.00 (μg/mL), the mark was set at 0.2 (μg/mL) in the figure

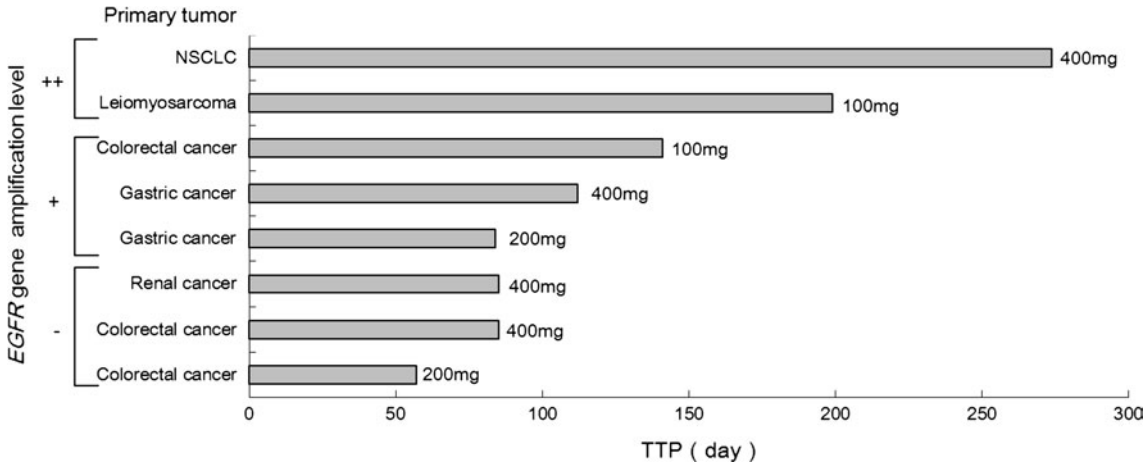

Fig. 4 Time to progression by *EGFR* gene copy number level

bevacizumab or trastuzumab. The relatively short $t_{1/2}$ may suggest weekly dosing is necessary for nimotuzumab. If nimotuzumab is used in combination with other chemotherapy which has long $t_{1/2}$, the dosing schedule should be set with careful consideration.

PD analysis on EGFR and downstream signaling pathway components was conducted using skin tissue at pre- and on-treatment in order to investigate a biological effective dose. There was no correlation between the changes of molecules and nimotuzumab doses. These findings were similar to the previous phase I studies [10, 16]. Considering the thesis of biding property of nimotuzumab [14, 15], skin tissue may not be appropriate for PD analysis. It might be preferable to use tumor tissue for the future PD study.

The *EGFR* gene copy number status was measured by FISH method with 8 patient's tumor tissues. It was considered that TTP correlated with the FISH score positively. Consistent with our results, previous study has shown that an increased *EGFR* gene copy number detected by FISH was associated with better outcomes in non-small cell lung cancer patients receiving chemotherapy with cetuximab [25]. In addition, the recent clinical study of nimotuzumab with Japanese and Korean gastric cancer patients reported the positive correlation between efficacy, and both EGFR protein expression level and gene copy number status [20]. Based on the theory that bivalent binding is required to establish stable attachment of nimotuzumab to the cellar surface, high EGFR surface density may be desirable for

pharmacological action of nimotuzumab. However, no correlation between efficacy and EGFR expression level was found in this study. *EGFR* gene copy number is reported to correlate with EGFR protein expression on the cell surface [27]; however, our study exhibited that the FISH score was not correlated with EGFR expression level determined by IHC as shown in Online Resource 3. The possible reasons for the discrepancy of results between FISH and IHC seem to be the variety of the tumor types, fixation method, and condition of tumor samples. Further investigation into the predictive biomarker of nimotuzumab is warranted in the future study.

In conclusion, nimotuzumab administered weekly is well tolerated up to 400 mg in Japanese patients. These results support that 400 mg weekly administration might be recommended for further clinical studies, especially for nimotuzumab monotherapy. The property of low dermatological toxicity could be a preferable profile as an anti-EGFR mAbs. Our results support the further evaluation of nimotuzumab including biomarker exploration in the following phase II studies.

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